

Appl. No. 09/699,667

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application;

Claims 1 to 30 (canceled)

Claim 31 (currently amended): A method for cleaving a nucleic acid substrate with a nucleic acid enzyme comprising mixing said substrate with the nucleic acid enzyme of claim + 44.

Claim 32 (previously presented): The method of claim 31, wherein the substrate is composed of ribonucleotides.

Claim 33 (previously presented): The method of claim 31, wherein the substrate is composed of a mixture of ribonucleotides and deoxyribonucleotides.

Claims 34 to 43 (canceled)

Claim 44 (new): A nucleic acid enzyme capable of recognizing and cleaving a nucleic acid substrate, said nucleic acid enzyme which when bound to the substrate comprises:

(i) a first nucleotide sequence

5'-G¹G²G³U⁴C⁵C⁶A¹³C¹⁴C¹⁵UC¹⁶C¹⁷UCGCG¹⁵G¹⁴U¹³N¹N²N³N⁴N⁵N⁶N⁷G¹⁷G¹⁶G⁷C⁸A⁹U¹⁰G¹¹C¹²S¹B¹Y-3' (SEQ ID NO: 65); and

(ii) a second nucleotide sequence

5'-B²K³S²G¹²C¹¹A¹⁰U⁹G⁸G⁷CUAAGG⁶G⁵A⁴C³C²C¹-3' (SEQ ID NO: 66);

wherein non-variable nucleotides having the same superscript form conventional Watson-Crick base pairs, except the two G⁷ form a homopurine base pair;

S¹ and S² are each independently selected from the group consisting of G and C;

B¹ and B² are each independently selected from the group consisting of G, C, U and T;

K is selected from the group consisting of G, U and T;

Appl. No. 09/699,667

Y is selected from the group consisting of C, U and T;

S¹ and S² form a conventional Watson-Crick base pair;

Y and B² may form a conventional Watson-Crick base pair;

B¹ and K may form a conventional Watson-Crick base pair;

B¹, K, Y and B² may together form a loop;

N¹N²N³N⁴N⁵N⁶N⁷ forms a substrate binding region;

N¹, N², N³, N⁴, N⁵ and N⁶ are each a nucleotide which may be the same or different;

N⁷ is U;

N⁷ is capable of forming a wobble pair with the substrate;

N¹, N², N³, N⁵ and N⁶ are capable of forming conventional Watson-Crick base pairs with the substrate; and

N⁴ is capable of forming a non-conventional Watson-Crick base pair and a conventional Watson-Crick base pair.

Claim 45 (new): The nucleic acid enzyme of claim 44, wherein said enzyme comprises a nucleotide sequence selected from the group consisting of:

(i) 5'-G¹G²G³U⁴C⁵C⁶A¹³C¹⁴C¹⁵UC¹⁶C¹⁷UCGCG¹⁵G¹⁴U¹³N¹N²N³N⁴N⁵N⁶N⁷G¹⁷G¹⁶G⁷C⁸A⁹U¹⁰G¹¹C¹²G¹⁸GCUUC¹⁸G¹²C¹¹A¹⁰U⁹G⁸G⁷CUAAGG⁶G⁵A⁴C³C²C¹-3'
(SEQ ID NO: 61); and

(ii) 5'-G¹(r²)G³U⁴C⁵C⁶A¹³C¹⁴C¹⁵UC¹⁶C¹⁷UCGCG¹⁵G¹⁴U¹³N¹N²N³N⁴N⁵N⁶N⁷G¹⁷G¹⁶G⁷C⁸A⁹U¹⁰G¹¹C¹²C¹⁸UUCGG¹⁸G¹²C¹¹A¹⁰U⁹G⁸G⁷CUAAGG⁶G⁵A⁴C³C²C¹-3'
(SEQ ID NO: 62).

Appl. No. 09/699,667

Claim 46 (new): The nucleic acid enzyme of claim 44, wherein said first nucleotide sequence is 5'-G¹G²G³U⁴C⁵C⁶A¹³C¹⁴C¹⁵UC¹⁶C¹⁷UCGCG¹⁵G¹⁴U¹³N¹N²N³N⁴N⁵N⁶N⁷G¹⁷G¹⁶G⁷C⁸A⁹U¹⁰G¹¹C¹²G¹⁸C¹⁹C²⁰-3' (SEQ ID NO: 63) and said second nucleotide sequence is 5'-G²⁰G¹⁹C¹⁸G¹²C¹¹A¹⁰U⁹G⁸G⁷CUAAGG⁶G⁵A⁴C³C²C¹-3' (SEQ ID NO: 64).

Claim 47 (new): The nucleic acid of claim 44, wherein N¹N²N³N⁴N⁵N⁶N⁷ is selected from the group consisting of:

- (i) CCGACCU;
- (ii) CCCAGCU;
- (iii) GGGAUAU;
- (iv) CCGCCCCU;
- (v) CCGGCCCCU;
- (vi) CCGUCCU;
- (vii) CCGAACU;
- (viii) CCGAGCU;
- (ix) CCGAUCU;
- (x) CCUCUUU;
- (xi) CCUUGUU;
- (xii) UGUUCUUU;
- (xiii) GGUGGUUU;
- (xiv) UCUCCUU;
- (xv) GGACUCU;

Appl. No. 09/699,667

(xvi) UCGACUU; and

(xvii) GCCACCU.

Claim 48 (new): The nucleic acid enzyme of claim 44, wherein the enzyme is derived from hepatitis delta virus.

Claim 49 (new): The nucleic acid enzyme of claim 44, wherein the substrate is composed of ribonucleotides.

Claim 50 (new): The nucleic acid enzyme of claim 44, wherein the substrate is composed of a mixture of ribonucleotides and deoxyribonucleotides.

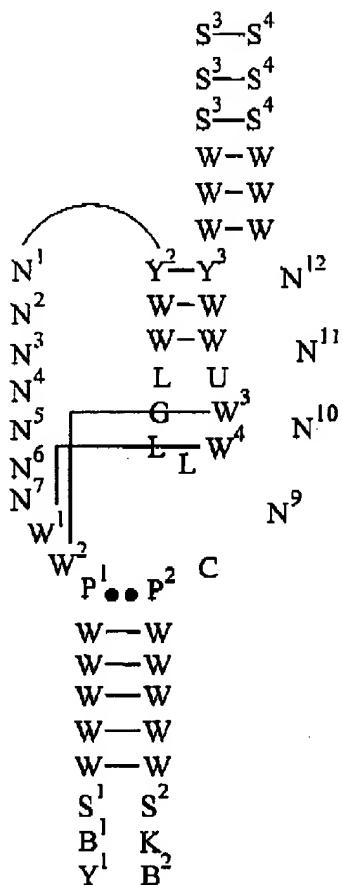
Claim 51 (new): The nucleic acid enzyme of claim 44, wherein the enzyme is composed of ribonucleotides.

Claim 52 (new): The nucleic acid enzyme of claim 44, wherein the enzyme is composed of a mixture of ribonucleotides and deoxyribonucleotides.

Claim 53 (new) The nucleic acid enzyme of claim 44, wherein the nucleotide residue of said substrate directly 5' to the cleavage site does not form a base pair with said enzyme.

Appl. No. 09/699,667

Claim 54 (new): A nucleic acid enzyme capable of recognizing and cleaving a nucleic acid substrate at a cleavage site, which when bound to the substrate comprises:



Y^2 and Y^3 together form a Watson-Crick base pair and are each independently selected from U, A and T;

S^3 and S^4 together form a Watson-Crick base pair and are each independently selected from U, A, G and C;

N^9 , N^{10} , N^{11} and N^{12} are nucleotides none of which form part of a Watson-Crick base pair;

P^1 and P^2 are the same purine nucleotide forming a homopurine base pair;

Appl. No. 09/699,667

W, W¹, W², W³ and W⁴ are each independently selected from any nucleotide that is part of a Watson-Crick base pair;

W¹ and W⁴ form a Watson-Crick base pair;

W² and W³ form a Watson-Crick base pair;

L are each independently selected from any nucleotide, each L is single stranded and does not form a Watson-Crick base pair;

one to two L may be absent;

Y¹ and B² may be joined to form a loop or may not form a loop but may form a Watson-Crick base pair;

S¹ and S² may form a Watson-Crick base pair;

B¹ and K may form a Watson-Crick base pair;

B¹, K, Y¹ and B² may together form a loop;

wherein S¹ and S² are independently selected from the group consisting of G and C;

B¹ and B² are each independently selected from the group consisting of G, C, A, U and T;

K is selected from the group consisting of C, A, G, U and T;

Y¹ is selected from the group consisting of G, A, C, U and T;

N¹N²N³N⁴N⁵N⁶N⁷ forms a substrate binding region;

N¹, N², N³, N⁴, N⁵ and N⁶ are each a nucleotide which may be the same or different;

N⁷ is U;

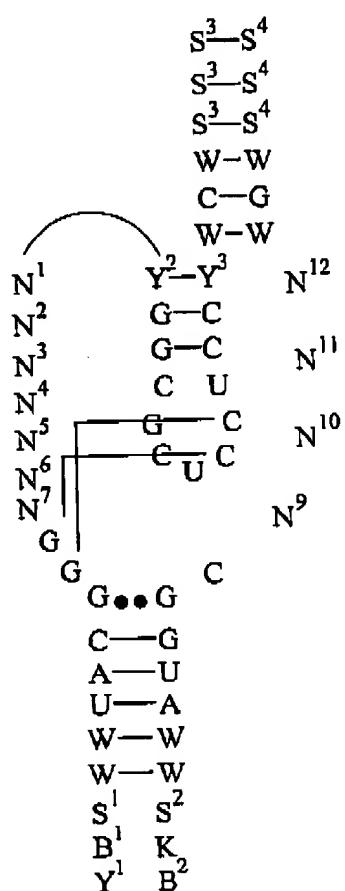
N⁷ is capable of forming a wobble pair with the substrate;

Appl. No. 09/699,667

N^1, N^2, N^3, N^5 and N^6 are capable of forming conventional Watson-Crick base pairs with the substrate; and

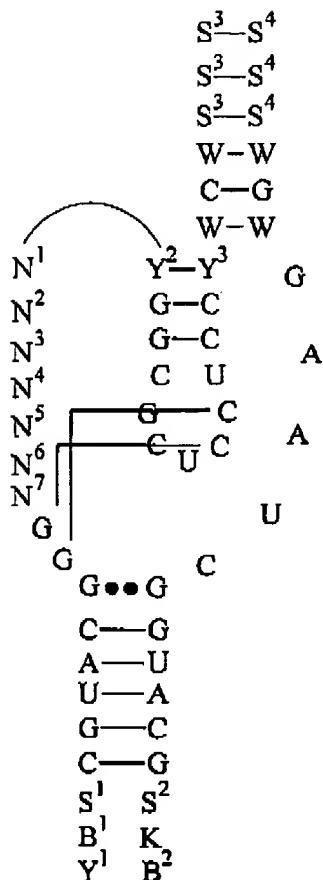
N^4 is capable of forming a non-conventional Watson-Crick base pair and a conventional Watson-Crick base pair.

Claim 55 (new): The nucleic acid enzyme of claim 54, wherein the enzyme is further defined as follows:



Appl. No. 09/699,667

Claim 56 (new): The nucleic acid enzyme of claim 54, wherein the enzyme is further defined as follows:



Claim 57 (new): The nucleic acid enzyme of claim 44, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.

Claim 58 (new): The nucleic acid enzyme of claim 45, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.

Appl. No. 09/699,667

Claim 59 (new): The nucleic acid enzyme of claim 46, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.

Claim 60 (new): The nucleic acid enzyme of claim 54, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.

Claim 61 (new): The nucleic acid enzyme of claim 55, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.

Claim 62 (new): The nucleic acid enzyme of claim 56, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.